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Morphology of a Sterile, Tetraploid, Hybrid Whiptail Lizard (Squamata: Teiidae: *Cnemidophorus*)

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ABSTRACT

Experimental hybridization with whiptail lizards has been conducted in order to improve understanding of the evolution of parthenogenesis in vertebrates and the effects of horizontal gene transfer in *Cnemidophorus*, the systematics of which has been confused owing to the reticulate phylogeny within the genus. Here we describe the external morphology and reproductive tissue histology of a sterile tetraploid hybrid between *C. sonorae* (triploid, unisexual) × *C. tigris* (diploid, bisexual), and compare her to her parents and siblings that developed from unfertilized eggs (normally cloned *C. sonorae*). This may help to identify F_1 hybrids that are found in nature and may help to determine whether they are sterile without conducting extensive laboratory breeding programs.

Considering that the maternal parent (*C. sonorae*) represented a clone that was of hybrid origin itself, the four genomes in the tetraploid hybrid historically were derived from three hybridization events among three bisexual species of *Cnemidophorus*, probably as follows: [(*inornatus* ♀ × *burti* ♂) × *burti* ♂] × *tigris* ♂. The tetraploid inherited 100% of its mother's genes and morphologically was very similar to her and her cloned offspring, particularly in scalation. Nevertheless, it was slightly larger than its siblings at hatching, grew faster than its siblings, attained a larger size, and, beginning at an age of six months, developed dorsal spots reflecting paternal traits in its color pattern. However, if this lizard had been found in nature, without any knowledge of its life history and in the absence of genetic data, it could easily have been misidentified as *Cnemidophorus exsanguis*, which it resembled more closely than its parental species.

Although she reached adult size and lived for more than two years beyond the age at which her cloned siblings produced offspring (nine months), the tetraploid never reproduced. Her ovaries were abnormally small, had poorly defined follicular epithelium with little vascularization, and had either empty or fluid-filled follicles devoid of oocytes. She also had numerous abnormally large mesonephric tubules and few or no cilia in the median oviduct. These traits should be examined in other specimens hypothesized to be sterile F_1 hybrid females.

INTRODUCTION

Individuals resulting from interspecific hybridization or those having genetic imbalances often are sterile, even if robust and healthy. Mules are classic examples. However, females of the unisexual species of whiptail lizards (genus *Cnemidophorus*) normally reproduce by parthenogenesis (Hardy and Cole, 1981), even though their lineages are of hybrid origin (for reviews, see Densmore et al., 1989; Dessauer and Cole, 1989; Moritz et al., 1989; Cole and Dessauer, 1993; Wright, 1993). Parthenogenetic lizards perpetuate the F_1 hybrid state generation after generation by cloning (Dessauer and Cole, 1986).

Some unisexual lineages consist of triploid lizards that historically originated through two hybridization events, yet they continue to produce viable triploid eggs and offspring by parthenogenetic cloning. The first hybridization was a mating between a male and female of different bisexual (dioecious; gonochoristic) species, resulting in a diploid F_1 female capable of cloning. The second hybridization event was the mating of one of these parthenogenetic females (which produce diploid eggs) with a male of a different species (either one of the original ancestral forms or a third bisexual species); this is followed by cloning of the new triploid female hybrid.

Not all hybridization leading to the triploid level results in viable clones; some apparently F_1 triploid hybrids that have been produced recently in nature have not produced continuing lineages (Christiansen and Ladman, 1968; Walker et al., 1989a, b, 1991). Nevertheless, the systematics of *Cnemidophorus* has long been plagued by confusion owing to frequent hybridization and horizontal gene transfer affecting reticulate phylogenies.

Tetraploidy is the highest level of ploidy known to be attained by any healthy lizard, but triploidy is the highest level of ploidy attained by any reproductive lizards (parthenogenetic clones). Only four genetically confirmed tetraploids of *Cnemidophorus* are known, including the one in the present report; each is the product of hybridization be-

tween a female of a triploid unisexual species and a male of a diploid bisexual species (Lowe et al., 1970; Neaves, 1971; Cole, 1979; Walker et al., 1990).

For the present report we examined the morphology (external and internal, gross and histological) of one of the four known tetraploid hybrids and compared this individual to its parents and siblings from cloned (unfertilized) eggs. We also address two basic questions concerning sterile hybrids, as follows: (1) Are there anatomical clues that can be applied without using laboratory culture techniques to determine whether or not suspected hybrids are sterile; and (2) can we improve understanding of the causes of sterility in certain hybrids? In addition, we hope to improve understanding of the evolution of parthenogenesis and polyploidy in vertebrates.

The tetraploid individual described here was the product of hybridization between the unisexual *Cnemidophorus sonorae* × *Cnemidophorus tigris marmoratus*, a diploid bisexual species. *Cnemidophorus sonorae* is a triploid clone (Dessauer and Cole, 1986) with an ancestry that probably involved two diploid bisexual species: (1) initially, *Cnemidophorus inornatus* (♀) × *Cnemidophorus burti* (♂) (Densmore et al., 1989); and (2) the resulting clonal diploid parthenogen (♀) × *C. burti* (♂) (Dessauer and Cole, 1989).

A third hybridization event (in the laboratory) between *C. sonorae* and *C. t. marmoratus* produced the tetraploid lizard described here. The correct identification of the tetraploid hybrid has been confirmed by genetic analyses of karyotypes (Cole, 1979) and of 29 structural genes analyzed by protein electrophoresis (Dessauer and Cole, 1984), including comparisons with the mother, father, and siblings. If this individual had been found in nature, in the absence of genetic information, it could easily have been misidentified as *Cnemidophorus exsanguis*, which it resembled more closely than its parental species (fig. 1B), and its biological significance may have been overlooked.

The tetraploid was sacrificed and preserved at an age of about 40 months, more than 2.5 years after attaining mature size and within months of attaining maximum laboratory longevity for the maternal species. She never laid eggs, although her mother and three of her normal, cloned sisters did so.

Acknowledgments

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MATERIALS AND METHODS

Laboratory Rearing

Lizards were maintained in captivity during the 1970s by methods for providing care, incubating eggs, and rearing offspring of *Cnemidophorus* described by Cole and Townsend (1977). Maintenance of healthy whiptails in captivity has been improved (Townsend, 1979; Townsend and Cole, 1985), although some mysteries remain to be solved for long-term maintenance of lineages generation after generation (Porter et al., 1994). The hybridization was accomplished by the lizards themselves, after the female *C. sonorae* was caged with sexually active males of *C. t. marmoratus*.

Specimens Examined

The laboratory-created tetraploid hybrid is AMNH (American Museum of Natural History specimen number) R-122989.

The mother of the hybrid is AMNH R-117812, a *C. sonorae* from Arizona: Cochise County; 7.1 km (linear) N and 4.2 km (linear) W Portal, 1494 m elev. We also examined six of her apparently normal, cloned F_1 daughters that were produced in the laboratory; three of these were confirmed genetically (AMNH R-117820–117822) and three were not (AMNH R-117816 and R-117828–29).

The father of the hybrid is a *C. t. mar-*

moratus, probably AMNH R-117811, from New Mexico: Hidalgo County; 15.6 km (by NM hwy. 90) NE Lordsburg, 1466 m elev. Although another male of this taxon was also caged at times with the mother, this was the only one seen to be sexually active while caged with her, and mating between him and the mother was frequently observed.

In order to examine possible male sexual influence on histological characters, comparative histological observations were made on two additional specimens of *C. tigris*, both in the Museum of Life Sciences, Louisiana State University in Shreveport (LSUS). One was a male *C. t. marmoratus* (LSUS 971) from Texas: Brewster County; 17.4 km E Panther Junction (Big Bend National Park) on Philips Ranch; the other a female *C. t. punctilinealis* (LSUS 1849) from New Mexico: Hidalgo County; 21.4 km (via hwy. 80) N Rodeo. Using representatives of these two subspecies is acceptable for the present purposes because these forms interbreed with panmixia in nature and they produce viable and normal, fecund offspring with allele frequencies in Hardy-Weinberg equilibrium (Dessauer and Cole, 1991 and in prep.).

External Morphology

Notes on coloration and size were recorded periodically for individual lizards as they grew up in the laboratory. Sex of each individual was determined by dissection and examination of ovaries or testes. Characters of scalation were discussed by Cole et al. (1988). The number of subdigital lamellae under the fourth toe was not counted because that toe was missing from most specimens. Subdigital lamellae beneath the fourth finger were counted only on the left finger because the right one was missing from many specimens.

Internal Morphology and Histology

We examined three specimens of *C. sonorae*: A sexually mature female (AMNH R-117812) collected in the field and two of her normal, cloned laboratory offspring (AMNH R-117820 and R-117829, the first of which was confirmed genetically as cloned). We also examined the tetraploid hybrid *C. sonorae* × *C. t. marmoratus* (AMNH R-122989).

After gross examination, the left metanephros, mesonephros, adrenal, ovary, oviduct, uterus, anterior urodaeum, posterior urodaeum, coprodaeum, proctodaeum, and distal end of the intestine were removed for histological study from the mother (serially sectioned, 284 slides) and the hybrid (including also the same tissues from the right side plus attached fat body; 252 slides). For the other two offspring (87 slides from AMNH R-117820; 57 slides from AMNH R-117829) we studied the same tissues as from the hybrid.

After temporary storage in 70% ethanol, the tissues were processed for an hour in each step following the tertiary butyl alcohol schedule in Weesner (1960). Each block of tissue was serially sectioned at 10 μm. For each lizard, the odd-numbered slides were stained in Ehrlich's hematoxylin and eosin (regressive method); the even-numbered slides were stained by the Mallory triple connective tissue technique (Pantin method; Presnell and Schreibman, 1997) combined with Ehrlich's hematoxylin (regressive method). Histological and anatomical terminology follows Hardy and Cole (1981).

RESULTS AND DISCUSSION

Reproduction

The P_1 maternal *C. sonorae* (fig. 1A) was collected as an adult in the field by Richard G. Zweifel on 4 August 1974. During her 18 months of captivity (she was preserved on 19 February 1976) she produced nine clutches of eggs (totaling more than 30 eggs) while caged with one or two males of *C. t. marmoratus* (fig. 1C).

Thirteen of the eggs hatched and five of the F_1 offspring grew to adult size. Initially, all hatchlings looked alike, suggesting that all were from unfertilized eggs. However, one developed atypical spots after several months (see Color Pattern, below); this proved to be the tetraploid hybrid (fig. 1B). Three of the other F_1 offspring that were genetically tested proved to have been cloned from the mother (Cole, 1979; Dessauer and Cole, 1984). Comparisons of the developing color patterns among all of the F_1 offspring

indicate that only one hybrid was produced and the other four that attained an age of six months or more, and a body length of at least 55 mm (snout-vent), were cloned. The capability of producing both uniparental offspring and biparental offspring was clearly demonstrated by the maternal *C. sonorae*, which represents a unisexual species.

The hybrid individual was in a clutch of four eggs. All four were developing for the first two months of incubation, at which time two ruptured in handling and died. A month later the remaining two hatched on the same day, and the hatchlings both appeared normal, but one died after three weeks and quickly became desiccated and largely devoured by crickets, so its remains were discarded.

Incubation periods may not have differed for the hybrid versus nonhybrid clutches. Nonhybrid clutches took from 78–87 days from oviposition to hatching in spring and autumn, and the hybrid's egg took 90 days to hatch in winter. Eggs were maintained at ambient room temperatures in the laboratory, so shorter development times probably reflect higher prevailing temperatures more than anything else.

Sterility of the Hybrid

Three of the hybrid's cloned sisters (from a different clutch) grew to adulthood and produced offspring. Each of these was karyotypically screened to prove it was cloned and each produced its first clutch at an age of 9–10 months and a body (snout-vent) length of 59–60 mm.

The hybrid lived in captivity for 40 months under conditions similar to her cloned sisters and attained a body length of 86 mm (85 mm after nearly 20 years preserved in ethanol; table 2), but she never laid eggs and never appeared gravid. We unsuccessfully attempted artificial insemination by depositing a sample of active, motile spermatozoa from a *C. tigris* into the cloaca of the hybrid at age 35 months. We thus concluded that the hybrid was sterile.

External Morphology

Color Pattern

There are several different pattern types and genetic clones of *Cnemidophorus sonorae* (Dessauer and Cole, 1989), although some herpetologists would refer to all or some of these as different but as yet unnamed species (for example, Wright, 1993). It is beyond the scope of this paper to discuss nomenclatural problems with clonal lizards of hybrid origin (for example, Cole, 1990); we refer to these lizards as a clone of *C. sonorae* because a more suitable name is not available.

The maternal *C. sonorae* represents a pattern clone in which typically the only light spots on the body of adults are small yellow spots superimposed on the yellow, beige, or cream light stripes (fig. 1A). Hatchlings and juveniles lack spots, and old adults may have a few yellowish-beige dots dorsally on the upper hind leg.

Upon hatching, the tetraploid hybrid appeared no different from 10 other F_1 offspring of the same mother and for which detailed color notes were recorded at hatching. In cloned (nonhybrid) offspring, the yellow spots superimposed on the paravertebral light stripes were emerging at an age of 10 months and body length of 59–65 mm. At 14 months of age and 65–68 mm body length, the light spots were clear and visible on all of them.

In contrast, beige light spots began emerging on the dorsum of the tetraploid hybrid at an earlier age—at six months (body length not recorded). These light spots were unlike those of the mother, as they were emerging within the brown dark stripes rather than superimposed on the light stripes. At nine months of age and a body length of 70 mm, these spots and additional cream and beige spots were evident (fig. 1B), emerging first on the posterior body, then anteriorly. Clarity of the light spots increased with age, and at 12 months (body length of 74 mm) small beige light spots were also present, superimposed on the paravertebral light stripes; these spots became yellow later in life, and the paravertebral light stripes broke up into a pattern of collinear light spots. The presence of light spots (or mostly bars) and absence of the paravertebral light lines are characteristic of the paternal parent of the hybrid (fig. 1C).

The similarity between the tetraploid hybrid and *C. exsanguis* is not surprising, as *C. exsanguis* and *C. sonorae* share one or two

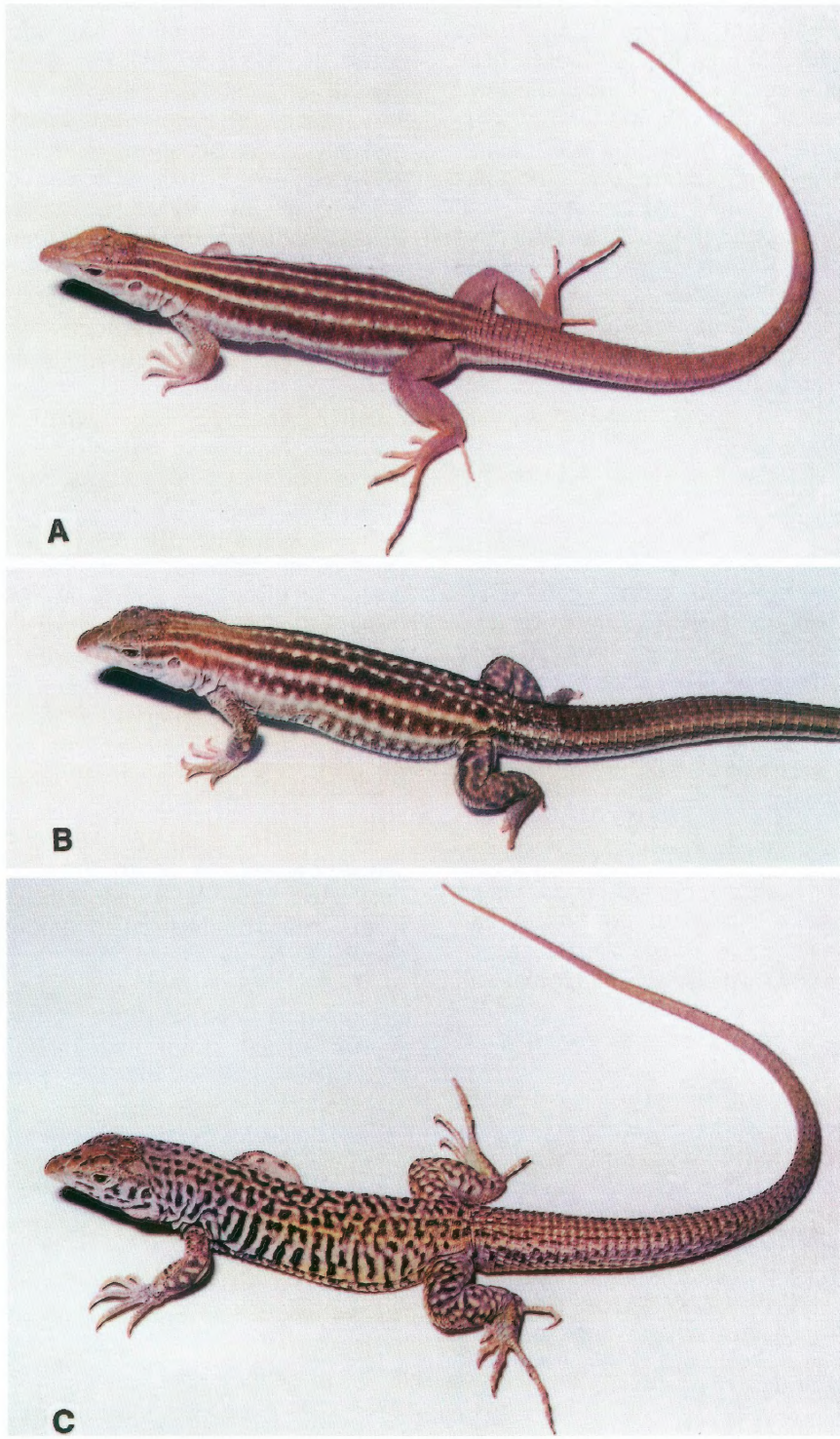


Fig. 1. Whiptail lizards of the genus *Cnemidophorus*. **A**. The maternal parent of the tetraploid hybrid, *C. sonorae* (AMNH R-117812; 25 February 1975, snout-vent length, ca. 79 mm). **B**. The tetraploid hybrid (*C. sonorae* × *C. tigris*; AMNH R-122989; 16 March 1978, snout-vent length, 90 mm). **C**. The probable paternal parent, *C. tigris* (AMNH R-117811; 26 April 1974).

ancestors among their separate hybrid origins (Dessauer and Cole, 1989). However, *C. tigris* was not involved in the origin of *exsanguis*, so the similarity in this instance seems convergent.

Growth

The maternal *C. sonorae* was preserved at a body length of 79 mm (perhaps actually 80 mm, since the measurement was made after 21 years in ethanol). Her age was at least 18 months, as she was an adult at capture (4 August 1974) and was preserved on 19 February 1976. However, it is conceivable that she was two years old when captured and three and a half when preserved.

Growth data for the tetraploid hybrid and three genetically confirmed cloned siblings are presented in table 1. The tetraploid was slightly larger at hatching and grew faster than did her cloned siblings. The fact that she grew to become larger than any other individual in the family is consistent with the paternal parent being larger than the maternal one (paternal influence; table 2), and the hybrid grew little after reaching 21 mo. of age (compare body length data in tables 1 and 2). Given that adults of the paternal taxon are typically larger than those of the maternal taxon, this negates concluding that the large size of the hybrid resulted primarily from being a tetraploid, presumably with larger body cells than a triploid has.

TABLE 1

Size and Growth Data for F_1 Offspring of *C. sonorae* AMNH R-117812

Age and data[a]	Nonhybrids[b]	Hybrid[c]
At hatching:		
SVL	31–32	33
Tail	58–59	63
Weight	0.8	0.9
9–10 months:		
SVL	59–65	70
Weight	6.0–8.0	9.2
12–14 months[d]:		
SVL	65–68	74
Weight	8.5–9.0	12.0
20–21 months:		
SVL	70–74	83
Weight	10.3–12.5	17.2
27–29 months:		
SVL	72–76	85
Weight	8.4	22

[a] Body length (snout-vent, SVL) is given in millimeters, as is tail length; weight is given in grams.

[b] Based on AMNH R-117820–117822.

[c] AMNH R-122989.

[d] Only the hybrid was 12 months old when these data were recorded, and she was larger than all of the others, which were 14 months old.

Scalation and Body Size

Scale characters were recorded for the hybrid, both parents, and six F_1 offspring of seven months age or older. The last six are cloned (nonhybrid offspring), as indicated by age and color pattern (see above) of those three that were not confirmed genetically.

In addition to the variable characters discussed below, we examined the following, which showed no differences among the specimens: position of nostril far anterior to nasal suture; frontoparietals 2; supraoculars 4 per side; parietals 3; and rows of enlarged ventrals across midbelly 8.

The hybrid resembles her mother and siblings but apparently differs from her father in nine of the 13 variable characters (table 2). However, for one of these 13 characters (finger lamellae), she differs from her father by only one scale.

The remaining four characters to be discussed are as follows: (1) in body length, the hybrid was intermediate between the mother and father (discussed above); (2) preanal scales were irregular (type III) in the hybrid and in the nonhybrid siblings, although both parents had type I, suggesting that the condition in the laboratory-reared offspring perhaps reflected the laboratory environment more than genetics (Dessauer and Cole, 1989); (3) some nonhybrid offspring showed asymmetry in arrangement of the cephalic plates, but the plates were symmetrical in the hybrid and both parents; and (4) the number of gulars in the hybrid was intermediate between her parents, but closer to the mother than to the father.

Summarizing the characters of external morphology, the hybrid was clearly intermediate between both parents in only the following: (1) dorsal pattern of spots; (2) body

TABLE 2

Size and Scalation Data for the Mother of the Hybrid, Cloned F_1 Offspring, the Hybrid, and the Father of the Hybrid

Character	Mother[a]	Nonhybrids[b]	Hybrid[c]	Father[d]
Snout-vent length (mm)	79	48–74	85	87
Mesoptychials abruptly enlarged?	Yes	Yes	Yes	No
Postantebrachials enlarged?	Yes	Yes	Yes	No
Preanal scales	Type I	Type III	Type III	Type I
Cephalic plates show bilateral symmetry?	Yes	No or yes	Yes	Yes
Gulars	16	14–15	17	20
Circumorbitals (total)	12	10–14	12	25
Scales around midbody	81	75–85	76	92
Interlabial scales (total)	16	15–18	16	27
Scales between paravertebrals	6	2–5	5	—
Femoral pores (total)	39	37–40	39	44
Scales between femoral pore series	2	2–4	2	5
Finger lamellae	15	14–15	15	16

[a] Based on AMNH R-117812.
[b] Based on AMNH R-117816, R-117820–R-117822, and R-117828–R-117829.
[c] Based on AMNH R-122989.
[d] Based on AMNH R-117811.

length; and (3) gulars. The hybrid was similar to her mother in all other characters. The cloned offspring were similar to their mother in all characters except that some individuals showed asymmetry in the cephalic scutes and all had type III preanals.

The hybrid was a tetraploid with three genomes from the mother and one from the father (Cole, 1979). Not only are 75% of its genes from its mother, but it also has 100% of the mother's genes. Only 25% of its genes are from the father, but it has only half of the father's genes. In addition, there are many genes in which the maternal and paternal species do not differ, so it is not surprising that the hybrid appears more like its mother than its father. This phenomenon was also discussed by Lowe et al. (1970), in the context of misidentification of hybrid males as specimens of unisexual species, when in fact they are actually polyploid F_1 hybrids.

Internal Morphology and Histology

The anatomy of the dissected viscera resembles that described for *Cnemidophorus exsanguis* by Hardy and Cole (1981). The maternal *C. sonorae* (AMNH R-117812) contained three enlarged ovarian follicles (one follicle is 5.5 × 6.4 mm) on the right and two on the left; all look normal. The adrenals and mesonephros appeared normal in all specimens examined. The tetraploid hybrid is a female with a normal oviduct and no hemipenes. However, no ovaries were seen during dissection (although small ones were found in the histological sections). Both nonhybrid siblings contained normal ovaries on both sides, containing follicles 1.2 to 1.5 mm in diameter. Relevant comparative data are presented in table 3.

The histological material was compared to the histological description for the triploid, parthenogenetic *C. exsanguis* (Hardy and Cole, 1981), and all of the same characters were examined. *Cnemidophorus sonorae* and *C. exsanguis* are similar in nearly all of these characters. Noticeable differences are described below (characters of *exsanguis* in parentheses). The maternal *sonorae* and two nonhybrid offspring are similar in most details; all three had normal yolked oocytes (fig. 3A), but the smallest one (AMNH R-117829), an immature individual, had an underdeveloped middle oviduct (table 3).

Cnemidophorus sonorae Compared to *C. exsanguis*

The following differences from *C. exsanguis* (in parentheses) may well result from ontogenetic or individual variation; addition-

TABLE 3
Size, Age, and Reproductive Characteristics of *C. sonorae* and the Tetraploid Hybrid (AMNH R-122989)

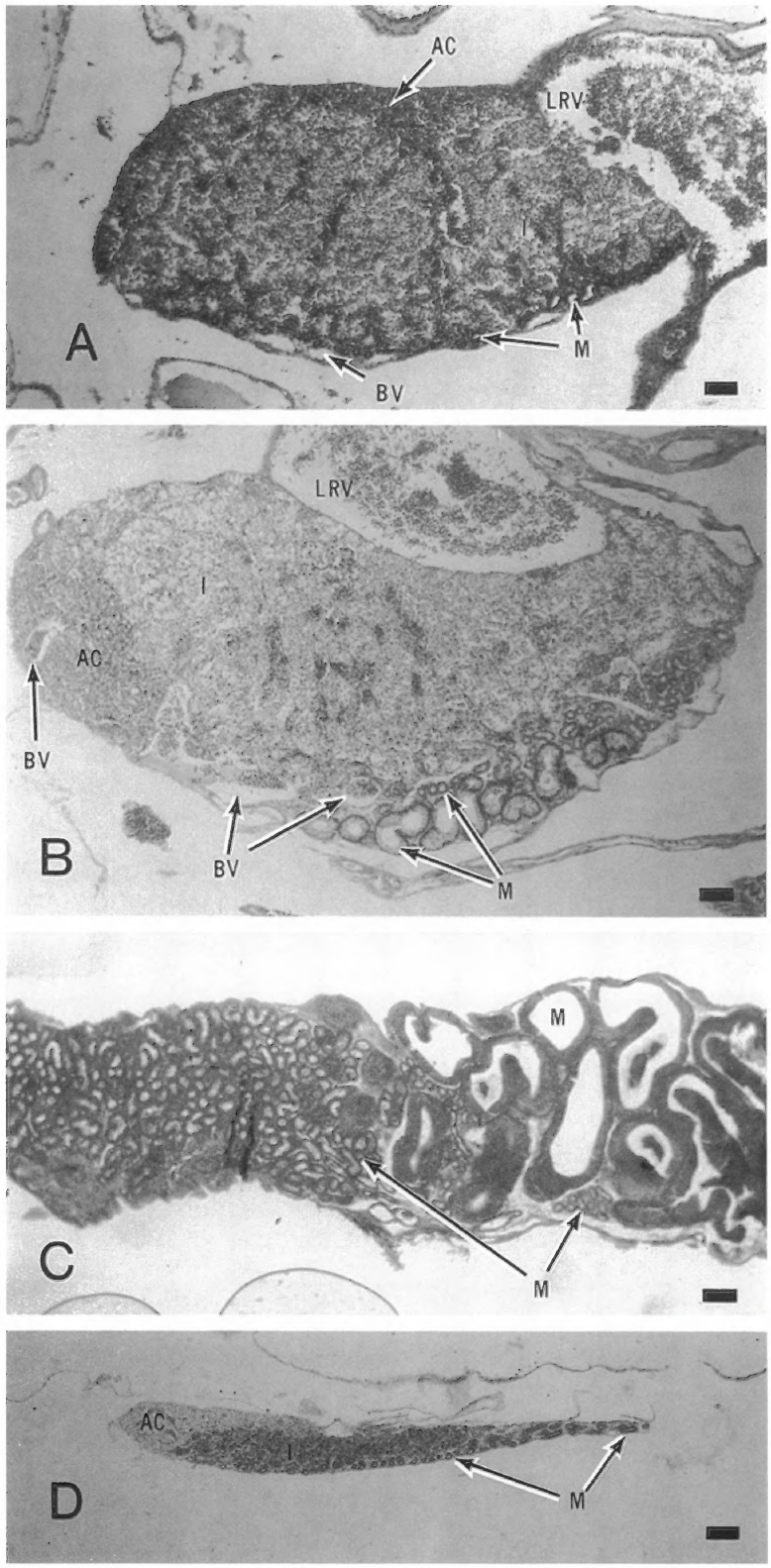
AMNH museum R-number	SVL (mm)	Age (mo.)	No. eggs laid	Follicles yolked	Middle oviduct glandular	Ovary width × length (mm)
117812	79	±30	>30	Yes	Yes	8.9 × 10.8
117820	64	15	±6	Yes	Yes	2.4 × 5.5
117829	48	7	0	Yes	No	1.8 × 4.0
122989	85	40	0	No	Yes	0.8 × 1.4

al study is needed to determine if they represent significant interspecific variation. The theca externa contains a well-defined theca granulosa, and the blood supply is abundant (fig. 3C). The mesonephros consists only of sparse small tubules (about 25 μm in diameter) on the opposite side of the adrenal gland from the adrenal cells (fig. 2A; in *exsanguis* mesonephric tubules are more abundant). No sperm are present. The basement membrane of the infundibulum is thin (in *exsanguis* it is thick). Seminal receptacles are absent (in *exsanguis* they are present). Absence of seminal receptacles probably represents a significant difference between the species and is consistent with Cuellar's (1968) report of their absence in 11 species of *Cnemidophorus*, although he incorrectly included *C. exsanguis* on that list. The theca granulosa of the largest follicle of R-117812 (fig. 3A, right) is at stage VIII with vitellogenesis advanced (see staging system developed for *Ctenosaura*; Uribe et al., 1996). The middle and left follicles in figure 3A are at stages IV and V, respectively.

The uterus enters the anterior urodaeum through the median side near the base of the poorly developed genital papilla in AMNH R-117812; it is the same but subapical to a well-developed genital papilla in AMNH R-117820, and the same but apical in AMNH R-117829 (in *exsanguis* the uterus opens through the apex of the well-developed genital papilla into the anterior urodaeum, which completely surrounds the genital papilla). The theca externa contains cuboidal-squamous epithelium in AMNH R-117812 and R-117820, but it is only cuboidal in R-117829 (in *exsanguis* it is squamous). The anterior urodaeum epithelium is stratified cuboidal to squamous (in *exsanguis* it is stratified cuboidal to columnar). The middle oviduct contains alveolar glands and the oviduct lumen contains ciliated, simple cuboidal epithelium in R-117812 and nonciliated, simple cuboidal-columnar epithelium in R-117820 (in *exsanguis* it is simple cuboidal-squamous and irregularly ciliated). However, in R-117829 no alveolar glands are present in the middle oviduct and cilia are rare (the oviduct is probably immature). The proximal oviduct is nonciliated and contains deep complex folds in R-117812, it is ciliated with shallow folds in R-117820, and nonciliated without folds in R-117829 (in *exsanguis*, it is ciliated with deep subdivided folds). The remnant of an encapsulated aborted and atretic ovum is between the metanephros and the posterior urodaeum in AMNH R-117812.

Tetraploid Hybrid Compared to Fecund *C. sonorae*

The hybrid attained adult size and mature age and contains somewhat enlarged ovarian follicles, but she was sterile (see above). The histology of its reproductive tract differs from that of its mother, a mature and fecund adult, in the following conspicuous ways. The follicular epithelium is incomplete, poorly defined, and irregular with more connective tissue, more vacuoles, and less vascularization (fig. 3C, D). The follicles (fig. 3B) are empty (or contained fluid) inside the theca granulosa and no oocytes are present in any secondary or tertiary follicles. The theca granulosa is an incomplete monolayer similar to stage II, even though at its approximate length of 0.8 mm (fig. 3B) the granulosa should be at stage IV or more of its ontogeny (Uribe et al., 1996). The mesonephros is more closely associated with the adrenal tissue (basically on the same side of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the adrenal gland and separated from the adrenal cells by a large blood vessel, probably the adrenal artery). There are many larger mesonephric tubules (up to 100 μm in diameter, or four times the diameter of the smaller tubules; fig. 2B) located close to the adrenal cells. Glandular tissue, normally found in the middle oviduct, is also present in the distal oviduct (the boundary between the middle and distal oviducts is abrupt and distinct in all specimens of *Cnemidophorus* we have examined). The glandular layer of the middle oviduct is about the same thickness as the glandular layer of the middle oviduct in *C. sonorae*, but the muscularis of the hybrid is much thinner (almost nonexistent) than in *sonorae* (fig. 3E, F). The completely ciliated uterus enters the anterior urodaeum through the apex of the genital papilla as it does in one sibling (AMNH R-117829); however, in the other sibling (AMNH R-117820) the uterus entrance is not apical.

The following characteristics of the tetraploid hybrid probably indicate its sterility. The ovarian follicles are abnormal (loosely organized, the follicle cell layer is incomplete, with more connective tissue, more vacuoles, and little vascularization) and they do not contain oocytes (fig. 3B, D); this apparent sterility is not a result of immature age because the lizard was more than three years old and its siblings had reproduced before the age of one year (see above). The infundibulum lacks seminal receptacles. The distal and proximal oviducts have thin mucosae and poorly developed folds. Some median oviduct alveolar glands are in the distal oviduct. The median oviduct has few or no cilia. The completely ciliated uterus and perhaps other characteristics, while different from the condition reported for *C. exsanguis* (Hardy and Cole, 1981) and other lizards in the present report, may not be related to sterility, but may instead reflect normal individual variation in the histological structure of reproductive tracts. The main source of estrogens is the theca interna of the ovary (Ozon, 1972; Guraya, 1965), which becomes hypertrophied during estrus (Fox, 1976). Since the hybrid contains a very thin theca interna it is probable that ovarian estrogen production was weak, at best.

Another character that might indicate sterility of the hybrid is the presence of mesonephric tubules that are intermediate in size between those of the maternal *C. sonorae* and an adult male of *C. tigris* (LSUS 971). In the male the mesonephros is very large and includes many large tubules (8–10 times larger in diameter than the smaller tubules); the smaller tubules of the male are about the same size as the smaller tubules of the hybrid and the average tubules of the mother and nonhybrid adult sibling (fig. 2). The enlarged mesonephric tubules of this hybrid probably reflect its sterile, hybrid state because such enlarged tubules were not found in other juveniles or adults of the other unisexual species we have examined.

Finally, we compared the mesonephros of the hybrid with that of a female *C. tigris* (LSUS 1849; fig. 2D). The mesonephric tubules of the female *tigris* are sparse and small (about 25 μm in diameter; fig. 2D), similar to those in *exsanguis* and *sonorae*, including the maternal parent of the hybrid and her nonhybrid adult sibling. We conclude that the enlarged mesonephric tubules in the hybrid reflect a genetic-developmental imbalance resulting from the hybridization.

It is intriguing that the enlarged mesonephric tubules of the hybrid tend toward the condition of maleness, as males have large mesonephric tubules that develop into the epididymydes (Fox, 1976; fig. 2C). One might thus say that the hybrid tended toward intersexuality. However, the hybrid inherited the X chromosome of *C. tigris*, not the Y (Cole,

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Fig. 2. Mesonephric kidneys and adrenal glands (hematoxylin and eosin stains). **A.** The maternal parent *C. sonorae* (AMNH R-117812; slide 45, section 2). **B.** Tetraploid hybrid (AMNH R-122989; slide 87, row 2, section 1). **C.** Adult male *C. tigris* (LSUS 971; slide 4, row 2, section 3). **D.** Adult female *C. tigris* (LSUS 1849; slide 17, section 1). Abbreviations are as follows: AC, adrenal cells; BV, blood vessel; I, interrenal cells; LRV, left renal vein; M, mesonephros (pointers indicate extreme diameters of mesonephric tubules). Scale bar, 0.1 mm.

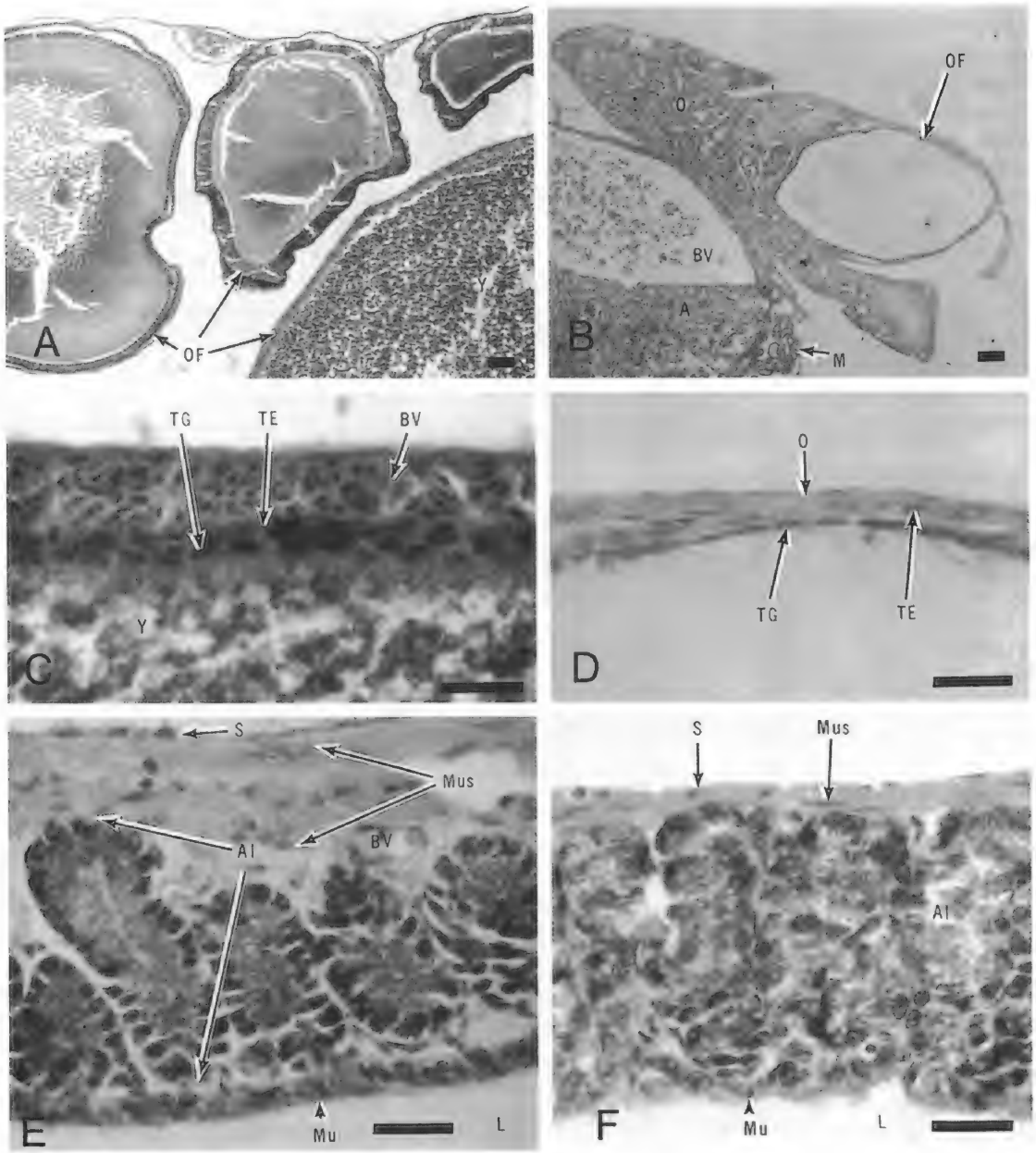

Fig. 3. Ovary and middle oviduct of the maternal parent of the tetraploid hybrid, *C. sonorae* (AMNH R-117812, A, C, E), compared to the tetraploid hybrid (AMNH R-122989, B, D, F); hematoxylin and eosin stains. **A**. Portion of the ovary, showing three immature follicles prior to vitellogenesis (left to right) and a more mature follicle with accumulating yolk granules (lower right) (slide 53, section 1). **B**. Small ovary of hybrid, with one enlarged follicle that does not contain an oocyte (slide 70, row 1, section 1). **C**. Detail of the follicle wall of the maternal parent (slide 53, section 1). **D**. Detail of the follicle wall of the hybrid (slide 69, row 2, section 1). **E**. Middle oviduct of the maternal parent (slide 193). **F**. Middle oviduct of the hybrid (slide 89, row 1, section 2). Abbreviations are as follows: A, adrenal gland; Al, alveolar glands; BV, blood vessel; L, lumen of middle oviduct; M, mesonephros; Mu, mucosa of middle oviduct; Mus, muscularis of middle oviduct; O, ovary; OF, ovarian follicle; S, serosa of middle oviduct; TE, theca externa of ovarian follicle; TG, theca granulosa of ovarian follicle; and Y, yolk granules in oocyte. Scale bar, 0.1 mm.

1979), so the gene or genes being expressed in this phenomenon probably are on the autosomes (see below), although not normally expressed as such in females.

Hybridization, Sex, and Sterility in *Cnemidophorus*

Interbreeding among bisexual taxa of whiptail lizards may produce: (1) no adult offspring whatsoever; (2) sterile offspring; (3) F_1 female hybrids capable of cloning and establishing a parthenogenetic lineage; or (4) fertile individuals of both sexes in panmictic hybrid zones. The outcome is in part a function of degree of relatedness of the interbreeding forms (Moritz et al., 1989; Cole and Dessauer, 1995).

The sterile tetraploid reported here has three genomes from the *sexlineatus* species group of the genus, inherited from the maternal *C. sonorae* (*inornatus* × *burti* × *burti*) and the fourth genome from the distantly related *tigris* species group. There have been other instances in which interbreeding between members of the *sexlineatus* and *tigris* species groups have produced clonal lineages, including reproductive diploids and triploids (for example, *C. neomexicanus*, *C. neotesselatus*; for reviews see Dessauer and Cole, 1989; Cole and Dessauer, 1995; Walker et al., 1997). It is possible that sterility in the tetraploid discussed here results more from genetic dysfunction at the tetraploid level than from hybridization. If this tetraploid hybrid were to attempt cloning eggs in the same fashion apparently used by her mother, with a premeiotic endoduplication followed by synapsis of sister replicate chromosomes in bivalents followed by the two cell divisions of meiosis (Uzzell, 1970; Cuellar, 1971), her primary oocytes would have had to function normally as octoploid cells containing 184 chromosomes. Even so, Neaves (1971) reported that a genetically confirmed tetraploid female hybrid *C. exsanguis* × *C. inornatus* laid two eggs that became desiccated in captivity, but that tetraploid individual had genomes from strictly within the *sexlineatus* species group.

The only tetraploid *Cnemidophorus* for which meiosis has been studied was a *C. sonorae* × *C. tigris punctilinealis* male that was found in nature (Lowe et al., 1970). Although spermatogonia and primary spermatocytes of that male were undergoing mitosis and meiosis I, it was not clear whether viable spermatozoa were being produced. Chromosomal bivalents and polyvalents were present, suggesting that genetic imbalances may have occurred in spermatozoa if any were produced.

Cnemidophorus tigris and its closest relatives are the only taxa in the genus with heteromorphic sex chromosomes (Cole et al., 1969; Bull, 1978). Males have one X and one Y chromosome; females have two X chromosomes. We suspect that maleness in whiptail lizards is determined actively by a gene or genes on the Y chromosome, for several reasons. First, the X chromosome (and absence of the Y) is seen in all diploid parthenogenetic clones for which *C. tigris* was one of the bisexual ancestors (for example, *C. neomexicanus*; Cole et al., 1988). Second, if females of bisexual species are XX, then females of unisexual species probably are also. Third, F_1 triploid hybrids between unisexual and bisexual species result in individuals of either sex (for example, Christiansen et al., 1971; Walker et al., 1989a), suggesting the addition of either one Y or a third X to the genome. Fourth, F_1 tetraploid hybrids between unisexual species and bisexual species similarly result in individuals of either sex (for example, Lowe et al., 1970, reported two males of *C. sonorae* × *C. tigris*, and a female is discussed here).

SUMMARY AND CONCLUSIONS

1. A female of a triploid unisexual species of whiptail lizard, *C. sonorae*, was maintained in captivity with a sexually active male of a bisexual species, *C. tigris*.

2. Two types of offspring were produced after mating was observed.

3. Most of the offspring were triploids, cloned by the mother from unfertilized eggs, but one tetraploid hybrid female offspring was produced also.

4. Considering that the origin of the maternal triploid clone of *C. sonorae* was ultimately from two prior hybridization events historically, the tetraploid hybrid had haploid genomes derived from three bisexual species

of *Cnemidophorus*, probably as follows: [(*inornatus* ♀ × *burti* ♂) × *burti* ♂] × *tigris* ♂.

5. The tetraploid hybrid was slightly larger than the nonhybrids at hatching, but otherwise appeared similar to normal hatchlings of *C. sonorae*.

6. The tetraploid grew faster than its nonhybrid siblings, and it attained a larger size than did they or their mother, approximating the larger size of its father.

7. In scalation the tetraploid hybrid appeared most similar to its mother and nonhybrid siblings, which is not surprising because 100% of their genes were present in the hybrid.

8. The tetraploid developed a distinctive dorsal color pattern of light spots that distinguished it from its mother and nonhybrid siblings, beginning at an age of six months. Consequently, the hybrid became rather similar to *C. exsanguis*, a species for which it could have been misidentified.

9. The tetraploid had inherited the X chromosome of the paternal *C. tigris* and was a female.

10. The nonhybrid F_1 siblings first reproduced at an age of 9–10 months and a snout-vent length of 59–60 mm.

11. The tetraploid was apparently sterile; she lived for 40 months, attained a snout-vent length of 86 mm, but never reproduced.

12. In gross dissection the tetraploid appeared to be nearly a normal female, but her abnormally small ovaries were not seen until we examined the histological sections.

13. Anatomy and histology of the reproductive system and associated organs of *C. sonorae* are similar to those of *C. exsanguis*, another triploid unisexual species in the *sexlineatus* group of *Cnemidophorus*.

14. Histology of the tetraploid was distinctive, however, in having a poorly defined follicular epithelium with little vascularization in the very small ovaries; empty or fluid-filled follicles devoid of oocytes; numerous abnormally large mesonephric tubules; and few or no cilia in the median oviduct.

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